

Anti-Platelet Drugs Block Platelet Activation by Vaccine-Induced Immune Thrombocytopenia and Thrombosis (VITT) Patient Serum

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Supplemental Information

Methods

Patients and Ethical approval

Patients presenting with thrombosis and thrombocytopenia, occurring after AZD1222 vaccination were recruited. Ethical approval for collecting blood from patients was approved under research ethics 15/NW/0079, and from healthy volunteers by Birmingham University Internal Ethical Review (ERN_11-0175). Samples from AZD1222 vaccinated individuals who did not develop VITT were collected as part of the COCO study, approved by the London - Camden and Kings Cross Research Ethics Committee (reference 20/HRA/1817).

Antibodies and reagents

Mouse monoclonal IgG2b antibody against human CD32 (IV.3) was purified from hybridoma cells supernatant, and IV.3 F(ab) fragment made using Pierce Fab Preparation kit (Thermo Fisher Scientific). Eptifibatide was from GSK. Ibrutinib, R406, and entospletinib were from Selleckchem. Rilzabrutinib was provided by Principia BioPharma. All other reagents were from Sigma-Aldrich.

Serum preparation

Serum was collected following centrifugation (2000×g, 10 minutes, room temperature [RT]) of clotted whole blood. Patient sera was collected before and after treatment with dexamethasone, IVIg and plasma exchange (see Table 1).

Human platelet preparation

Acid citrate dextrose (1:10, v/v) was added citrated blood taken from healthy, drug-free volunteers and centrifuged (200×g, 20 minutes, RT). Platelet rich plasma isolated and centrifuged with 0.2µg/mL prostacyclin (1000×g, 10 minutes, RT). Platelets were washed again in modified-Tyrodé's-HEPES buffer and prostacyclin, before resuspension in modified-Tyrodé's-HEPES and rested before testing.

Light transmission aggregometry (LTA)

Aggregation was measured using a light transmission aggregometer (Model 700, ChronoLog) for 10 minutes, 1200 rpm, 37°C following washed platelet stimulation with serum (15:1, v/v). IV.3 F(ab) and inhibitor pre-incubation was for 5 and 10 minutes respectively. Aggregations were conducted in washed platelets from 4 healthy donors

known to respond.

Statistical analysis

All data presented as mean \pm SEM, $p < 0.05$ was considered statistically significant. Statistical analysis was performed in GraphPad Prism 9 using one or two-way ANOVA with Dunnett corrections for multiple comparisons.